

PB86-209822

Genetic Activity Profiles of Chemicals
Selected from the Aneuploidy Data Base

Oak Ridge National Lab., TN

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1986



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA		
(Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-86/085	2.	3. RI PB86-209822
4. TITLE AND SUBTITLE Genetic Activity Profiles of Chemicals Selected from the Aneuploidy Data Base (Journal Version)		5. REPORT DATE 1986
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Michael D. Waters, H. Frank Stack, Kathleen H. Mavournin and Vicki L. Dellarco		8. PERFORMING ORGANIZATION REPORT NO. -
9. PERFORMING ORGANIZATION NAME AND ADDRESS Oak Ridge National Laboratory Env. Mutagen, Carcinogen & Teratogen Info. Program Oak Ridge, TN 37830		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. IAG#DW899-30922 Contract#68-02-3839
12. SPONSORING AGENCY NAME AND ADDRESS Office of Health & Environmental Assessment (RD-689) Reproductive Effects Assessment Group U.S. Environmental Protection Agency Washington, D.C. 20460		13. TYPE OF REPORT AND PERIOD COVERED Final
		14. SPONSORING AGENCY CODE EPA/600/21
15. SUPPLEMENTARY NOTES One of 12 articles prepared by a committee and including an introductory and summary paper. These reports were published together in <u>Mutation Research</u> , 167 (1986). <u>Mutation Res.</u> , 167:171-188, January 1986		
16. ABSTRACT A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.		
17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
19. DISTRIBUTION STATEMENT Release to the public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 19
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

MTR 05013

Genetic activity profiles of chemicals selected from the Aneuploidy Data Base

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(Received 14 June 1985)

(Accepted 27 June 1985)

Introduction

The qualitative aspects of the Aneuploidy Data Base** have been summarized in Dellarco et al. (this issue). The purpose of the present paper is to address quantitative aspects of the data using the genetic activity profile method described by Garrett and coworkers (1984). This preliminary analysis is consistent with one of the ultimate goals of this endeavor: the quantitative comparison of evaluated data from diverse sources.

In the present analysis, dose information, where available, was obtained from the Aneuploidy Data Base and verified by reference to the original

publications. Either the lowest effective dose tested (LEDT) or the highest ineffective dose tested (HIDT) was recorded for each agent and bioassay system. The term 'dose' in the context of the present report does not imply consideration of exposure or treatment time and, therefore, can be considered synonymous with concentration. For each compound, the quantitative results in the test systems were represented by computer-generated bar graphs (genetic activity profiles). The x-axis unit values correspond to the various test systems, and the y-axis values are the logarithmically transformed LEDT or HIDT values.

The sequence and organization of bioassay systems plotted on the x-axis and corresponding bioassay codes and definitions are shown in Table 1. The bioassays are grouped according to the phylogenetic classes of test systems and where appropriate, phylogenetic categories have been separated into mitotic and meiotic assay systems. It may be noted that in *Saccharomyces*, *Aspergillus*, *Neurospora* and in *Drosophila* the induction of aneuploidy is detected by genetic tests; whereas in plant and mammalian systems, cytogenetic techniques are used to detect whole chromosome gain or loss.

Profile line height (the magnitude of each bar) is a function of the LEDT or HIDT, which is associated with characteristics of each individual test system, such as population size, cell-cycle kinetics, and metabolic competency. Thus, the de-

* Operated by Martin Marietta Energy Systems, Inc. under contract No. DE-AC05-80R 21400 with the U.S. Department of Energy.

The material described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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TABLE 1
ORGANIZATION OF ASSAY CODES FOR ANEUPLOIDY TESTS

Mitotic test systems	Meiotic test systems
A. <i>Saccharomyces</i> (genetic) SC4 = chromosome loss	SC1 = chromosome gain
B. <i>Aspergillus</i> (genetic) A1 * = euploid segregants — 3 types A2 * = euploid segregants — 1 or 2 types A3 * = euploid segregants — altered ratios A4 * = aneuploid products identified A5 * = aneuploid products inferred	
C. <i>Neurospora</i> (genetic)	NC1 = disomics
D. Plants (primary cytogenetic) PL1 = <i>Allium</i> — somatic PL2 = <i>Vicia</i> — somatic PL3 = <i>Hordeum</i> — somatic PL7 = other	
E. <i>Drosophila</i> (genetic) DM3 = postmeiotic — chromosome gain DM4 = loss — stage unspecified DM5 = gain — stage unspecified	DM1 = chromosome loss DM2 = chromosome gain
F. Mammalian cells in culture (cytogenetic) MC1 = rodent — primary — numerical MC3 = rodent — transformed — numerical MC5 = human — primary — numerical MC9 = other	H. Mammalian female germ cells in vivo (cytogenetic) MF1 = MII oocytes MF2 = pronuclei MF3 = preimplantation embryos MF4 = postimplantation embryos MF5 = F ₁ progeny
G. Mammalian somatic cells in vivo (cytogenetic) MS1 = lymphocytes — numerical MS3 = rodent — lymphocytes — numerical MS5 = bone marrow — numerical	I. Mammalian male germ cells in vivo (cytogenetic) MM1 = premeiotic — numerical MM2 = premeiotic — related MM5 = meiotic — related MM6 = postmeiotic — related MM7 = other

* The last character of the *Aspergillus* assay code indicated primary or secondary effects: 0, N/A; 1, primary; 2, secondary; 3, either or both.

tection limit of each test system is different, and across a given profile, responses will vary substantially. The profiles may be compared and analyzed only on the basis of common test results (i.e., where the same test systems were used to evaluate two or more of the chemicals under consideration). No effort was made to adjust or relate responses in one test system to those of another. Such adjustments are difficult unless directly comparable protocols are employed.

To accommodate both positive and negative responses on a continuous scale, doses were transformed logarithmically so that effective (LEDT)

and ineffective (HIDT) doses were represented by positive and negative numbers, respectively. The response or logarithmic dose unit (LDU_{ij}) for a given test system i and chemical j was represented by the expressions:

$$LDU_{ij} = -\log_{10}(\text{dose}), \text{ for HIDT values; } LDU \leq 0$$

or

$$LDU_{ij} = -\log_{10}(\text{dose} \times 10^{-5}), \text{ for LEDT values;}$$

$$LDU \geq 0$$

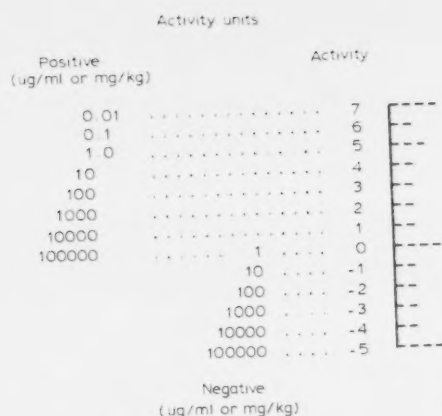


Fig. 1. Scale of logarithmic dose unit values.

These simple relationships define a dose range of 0 to -5 logarithmic units for ineffective doses ($1-100,000 \mu\text{g/ml}$ or mg/kg) and 0 to +7 logarithmic units for effective doses ($100,000-0.01 \mu\text{g/ml}$ or mg/kg). A scale illustrating the *LDU* values is shown in Fig. 1. Negative responses at doses less than 1 were set equal to 1. In effect an LEDT value $\geq 100,000$ or an HDT value ≤ 1 produces an *LDU* = 0; no useful information is gained from such extreme values.

For additional information on methods for determination of effective doses, representation of a genetic activity profile and data management and analysis, see Garrett et al. (1984).

Evaluation of compounds for induction of aneuploidy

The performance of assay systems was evaluated initially on a compound-by-compound basis. Compounds for which at least 3 different tests had been performed were selected for graphic presentation. To increase the data base on vinblastine, information on the free agent was combined with that for vinblastine sulfate. Those compounds for which there were at least 3 different test results are ranked in most-to-least-tested order in Table 2 with their Chemical Abstracts Services (CAS) Registry Numbers. The following is a descriptive assessment of the quantitative data on each of these compounds. Each assessment is accompanied by a

TABLE 2
ANEUPLOIDY DATA BASE

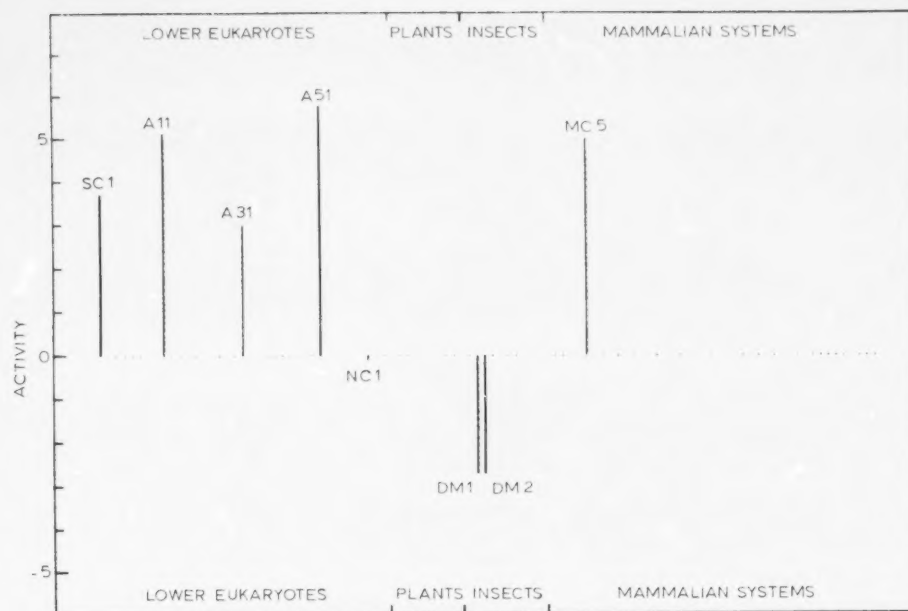
Chemicals (3 or more tests)	CAS Registry No.	Number of tests
Benomyl	17804-35-2	8
Cyclophosphamide	50-18-0	6
Triaziquone	68-76-8	6
Amitrole	61-82-5	4
Colchicine	64-86-8	4
Ethanol	64-17-5	4
Vinblastine	865-21-4	4
Vinblastine sulfate	143-67-9	
Actinomycin D	50-76-0	3
Benzo[<i>a</i>]pyrene	50-32-8	3
Bleomycin	11056-06-7	3
Caffeine	58-08-2	3
Chloral hydrate	302-17-0	3
Colcemid	477-30-5	3
DMSO	67-68-5	3
5-Fluorodeoxyuridine	50-91-9	3
<i>p</i> -Fluorophenylalanine	60-17-3	3
Griseofulvin	126-07-8	3
Halothane	151-67-7	3
Methyl methanesulfonate	66-27-3	3
Simazine	122-34-9	3

computer-generated profile and a coded listing of test results with a short citation. The test result or call is shown without/with exogenous metabolic activation. Dose values have been converted to $\mu\text{g/ml}$ for in vitro systems or to mg/kg for in vivo systems.

Benomyl

Benomyl is a carbamate that has been widely used as a systemic foliar fungicide. The compound displays very low acute toxicity ($> 10,000 \text{ mg/kg}$) by the oral route in mammals. In terms of test data acceptable by the committee, benomyl was the most fully evaluated compound.

Benomyl displayed positive results (Fig. 2) for chromosome gain in a meiotic assay in *Saccharomyces*. The induction of malsegregants was observed in mitotic cells of *Aspergillus* in 3 different tests. Despite the foregoing positive data, results were negative in a *Neurospora* meiotic assay for disomics. It should be noted, however, that the test in *Neurospora* was performed at a relatively low dose level ($1 \mu\text{g/ml}$). Meiotic assays for both



TEST CALL	DOSE	REFERENCE
SC1 +/0	20	SORA ET AL. 17, 1982
A11 +/0	0.80	MORPURGO ET AL. 81, 1979
A31 +/0	100	DE BERTOLDI & GRISELLI 303, 1980
A51 +/0	0.15	KAPPAS 189, 1978
A51 +/0	0.20	BIGNAMI ET AL. 395, 1977
NC1 -/0	1	GRIFFITHS 1980
DM1 -/0	500	LAMB & LILLY 83, 1980
DM2 -/0	500	LAMB & LILLY 83, 1980
MC5 +/0	1	TENCHINI ET AL. 139, 1983
MC5 -/0	20000	GUPTA & LEGATOR 1976

Fig. 2. Activity profile for benomyl.

chromosome loss and chromosome gain in *Drosophila* were negative at high dose equivalents (500 $\mu\text{g}/\text{ml}$). Conflicting positive and negative results were observed in mammalian cells in culture. The positive response (Fig. 2) was observed at a concentration equivalent of 1 $\mu\text{g}/\text{ml}$, however, a negative response was obtained with testing to a higher concentration equivalent (20 mg/ml).

It should be noted that benomyl frequently is found to be converted to or contaminated with its breakdown product methylbenzimidazole carbamate (MBC). This latter compound also has been

shown to induce aneuploidy in two test systems, chromosome loss in a mitotic assay in *Saccharomyces* (Wood, 1982; Zimmermann and Scheel, 1984; Zimmermann et al., 1984) and induction of malsegregants in a mitotic assay in *Aspergillus* (Kappas et al., 1974).

Colchicine

This alkaloid compound has been used medically in the treatment of gout. In view of its classical role as a spindle inhibitor in many test systems, it is surprising that the quantitative data

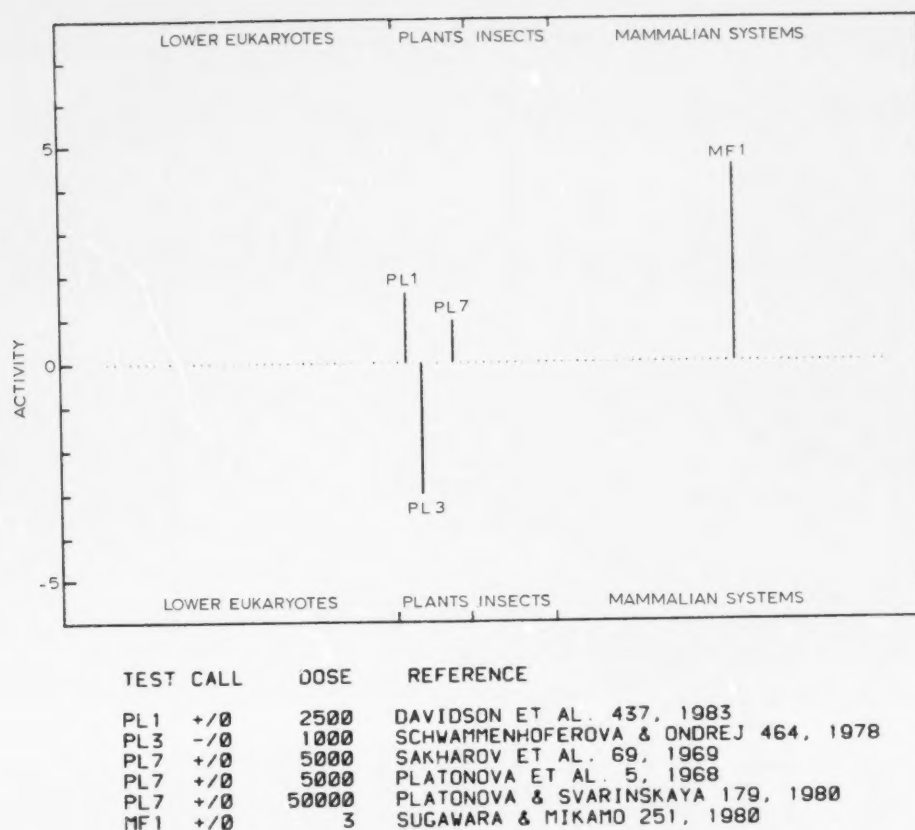


Fig. 3. Activity profile for colchicine.

base (Fig. 3) acceptable to the committee is extremely meager. And in plants, in fact, the data conflict. Somatic cell assays in *Allium* and in *Crepis* gave positive results at dose equivalents of 2.5 and 5 mg/ml, respectively, while a negative response was observed in *Hordeum* at an equivalent dose of 1 mg/ml. Positive effects were seen cytogenetically in metaphase II oocytes of Chinese hamsters at 3 μ g/ml.

Colcemid

Colcemid is an antineoplastic alkaloid that is structurally similar to colchicine but less toxic in most systems. It also is a well-tested inhibitor of tubulin polymerization. Data (Fig. 4) acceptable by the committee were obtained in *Drosophila* and

in mammalian cells in culture. Negative results were seen in a meiotic chromosome loss test at a relatively low dose (4 μ g/ml) in *Drosophila*, but a meiotic chromosome gain assay was positive; an overall positive call was recorded by the subcommittee. Positive results were obtained in several transformed rodent cell systems in vitro at LEDT values of 0.02–0.07 μ g/ml.

Vinblastine and vinblastine sulfate

These compounds are also antitumor alkaloids and classic spindle poisons. Results (Fig. 5) in a *Neurospora* meiotic assay for disomic chromosome gain were negative at 217 μ g/ml. In *Drosophila*, data negative for meiotic chromosome loss but positive for chromosome gain were re-

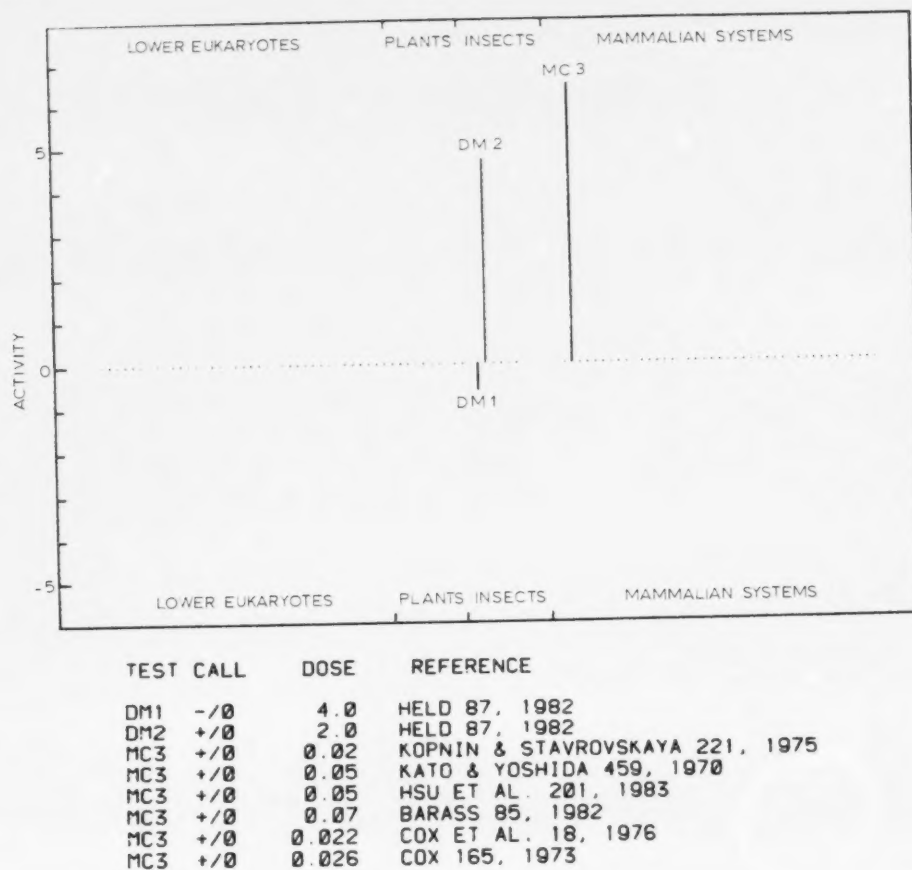


Fig. 4. Activity profile for colcemid.

ported at a common dose level of 100 $\mu\text{g}/\text{ml}$. Both results have been plotted. Numerical chromosome changes were observed at low doses (0.05–0.8 $\mu\text{g}/\text{ml}$) in cultured transformed cells.

Griseofulvin

Griseofulvin is an antifungal agent used in the treatment of athlete's foot. Positive results (Fig. 6) were obtained in 3 different species: 4 $\mu\text{g}/\text{ml}$ is the LEDT in a mitotic test for malsegregants in *Aspergillus*; at 150 $\mu\text{g}/\text{ml}$, the compound is positive in cultured cells in the plant *Medicago sativa*; mammalian cells in culture display a positive response to the compound at 40 $\mu\text{g}/\text{ml}$.

Chloral hydrate

Chloral hydrate or trichloroacetaldehyde is a metabolite of trichloro- and tetrachloroethylene, both of which are suspect human carcinogens. The compound itself is a sedative and an hypnotic. It appears to block pole-to-pole microtubule formation in addition to other effects on the spindle apparatus. As shown in Fig. 7, at a high concentration (3300 $\mu\text{g}/\text{ml}$ equivalent), in an *Aspergillus* mitotic test, the compound induces the formation of malsegregants. Aneuploid products have been observed in *Aspergillus* at a dose of 1650 $\mu\text{g}/\text{ml}$ equivalent. The compound is active in mice (82.7 mg/kg) in a meiotic assay for numerical chromosome changes in male germ cells.

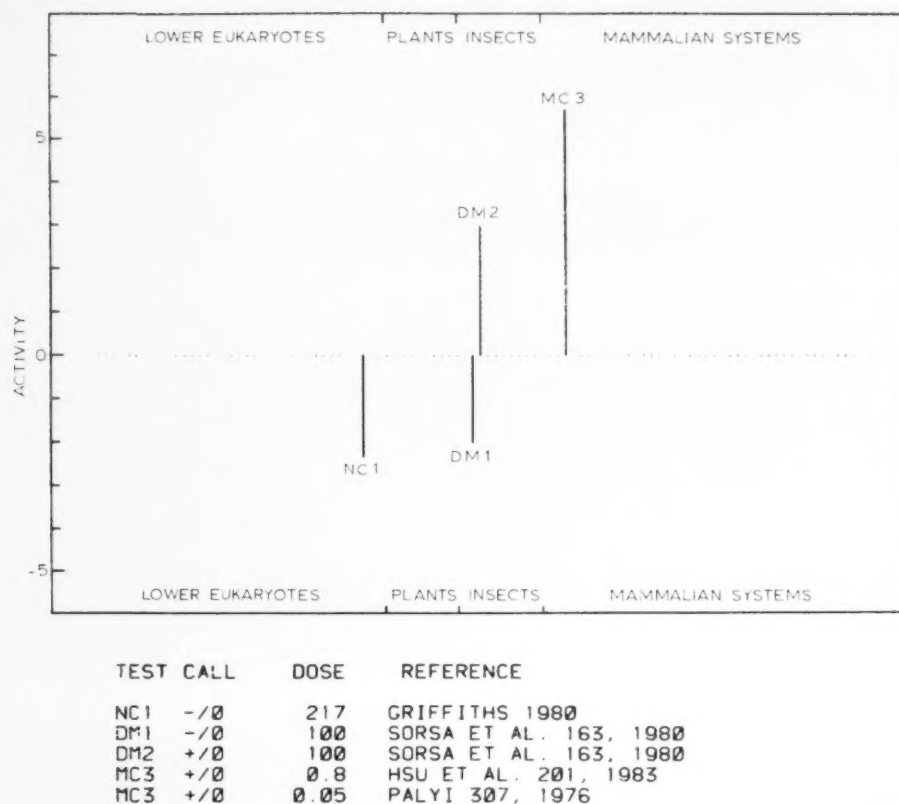


Fig. 5. Activity profile for vinblastine and vinblastine sulfate.

Actinomycin D

This antibiotic has been used as an antineoplastic agent. The compound directly inhibits nucleic acid synthesis and may secondarily affect centriole duplication. In mitotic assays in *Aspergillus* (Fig. 8) actinomycin D induces, at 12 $\mu\text{g}/\text{ml}$, the formation of aneuploid products (products inferred). In meiotic assays in *Drosophila*, the compound induces both chromosome loss and chromosome gain at doses equivalent to 10 $\mu\text{g}/\text{ml}$.

Triaziquone

This aziridine-substituted benzoquinone is a potent gene mutagen and clastogen in mammals. Surprisingly, therefore, it is inactive in *Drosophila* meiotic assays for chromosome loss and chromosome gain. Positive results in mice and negative

results in hamsters (Fig. 9) have been obtained in meiotic metaphase II oocytes at low dose equivalents (1.0 and 2.0 mg/kg, respectively). In unfertilized ova in mice, the compound produces positive results at 0.25 mg/kg. Similar results have been obtained in preimplantation embryos in mice. Interestingly, triaziquone gives negative results in hamster male germ cells at 0.25 mg/kg equivalent.

Halothane

Halothane is widely used as an anesthetic gas in humans. As shown in Fig. 10, it produces positive results for chromosome loss and chromosome gain in meiotic assays when fed to *Drosophila* at 3400 $\mu\text{g}/\text{ml}$. On the other hand, an aerosol dose equivalent of 79 $\mu\text{g}/\text{ml}$ is negative for meiotic nondisjunction in female mice.

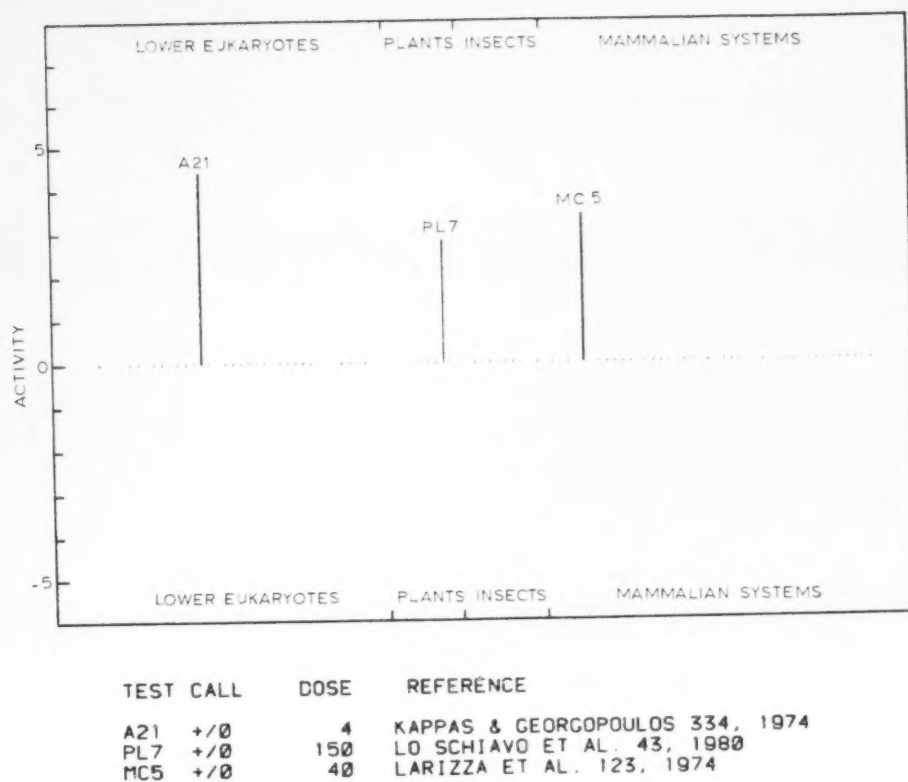


Fig. 6. Activity profile for griseofulvin.

para-Fluorophenylalanine

This amino acid analogue is incorporated into proteins, thus producing direct and indirect effects on mitotic as well as meiotic systems. It is positive in *Saccharomyces* at an undefined dose level. As shown in Fig. 11, the compound induces altered ratios of malsegregants and identifiable aneuploid products in *Aspergillus* at 183 and 3660 $\mu\text{g}/\text{ml}$, respectively. Disomics are induced in *Neurospora* at a low dose (1 $\mu\text{g}/\text{ml}$). *p*-Fluorophenylalanine, in fact, has been used as a positive control in the *Neurospora* assay.

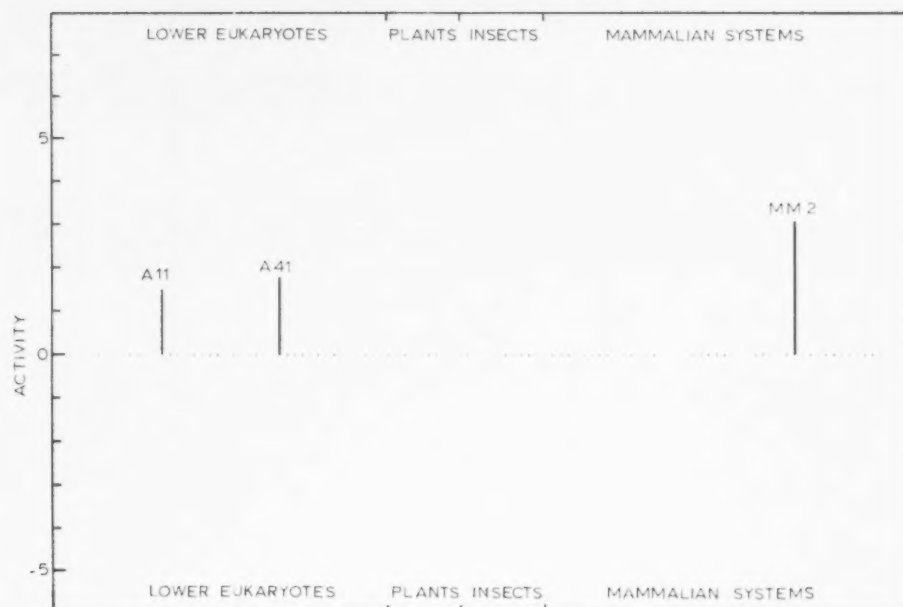
Dimethyl sulfoxide

DMSO is commonly used as a solvent for test agents in short-term in vitro assay systems. It is most often negative in genetic tests. DMSO (Fig.

12) failed to induce euploid segregants in mitotic assays in *Aspergillus* (at 4100 $\mu\text{g}/\text{ml}$), and produced negative results in meiotic chromosome loss and chromosome gain assays in *Drosophila* at 20 000 $\mu\text{g}/\text{ml}$.

Ethanol

Ethanol (Fig. 13) induces malsegregants and aneuploid products in mitotic tests in *Aspergillus* but only at levels of 24 000–40 000 $\mu\text{g}/\text{ml}$. Negative results were obtained in *Neurospora* at a similar dose (32 000 $\mu\text{g}/\text{ml}$). No reports in mammalian systems were accepted by the review committee. There is definitely a need for further studies on this compound in higher eukaryotic test systems.



TEST CALL	DOSE	REFERENCE
A11	+/-0	3300
A41	+/-0	1650
MM2	+/-0	82.7
		SINGH & SINHA 1976
		KAHER, 1985 (SUBMITTED)
		RUSSO ET AL. 695, 1984

Fig. 7. Activity profile for chloral hydrate.

Other genotoxic chemicals

Several additional compounds were tested in 3 or more assays with mostly positive results. Amitrole, benzo[a]pyrene, bleomycin, caffeine, cyclophosphamide, fluorodeoxyuridine, methyl methanesulfonate and simazine are examples of compounds that are generally genotoxic and may be considered non-specific inducers of aneuploidy. Activity profiles for these agents may be found in Waters et al. (1985).

Assessment of test system performance by phylogenetic class

LEDTs have been compared by rank order where the tests are grouped by phylogenetic category. For convenience, test results from Sac-

charomyces and higher plant systems are placed on the same graph (Fig. 14).

Saccharomyces

This system (Fig. 14) responds to agents, including methyl methanesulfonate and cyclophosphamide, that have been shown to be genotoxic in many other assays. Benomyl and its breakdown product MBC are both positive at approximately equivalent doses (10–20 µg/ml) in *Saccharomyces*.

Plants

Griseofulvin (Fig. 14), was found positive in cultured cells from *Medicago sativa*. Colchicine gives positive results in *Allium* and in *Crepis capillaris* but negative results in *Hordeum*. Since

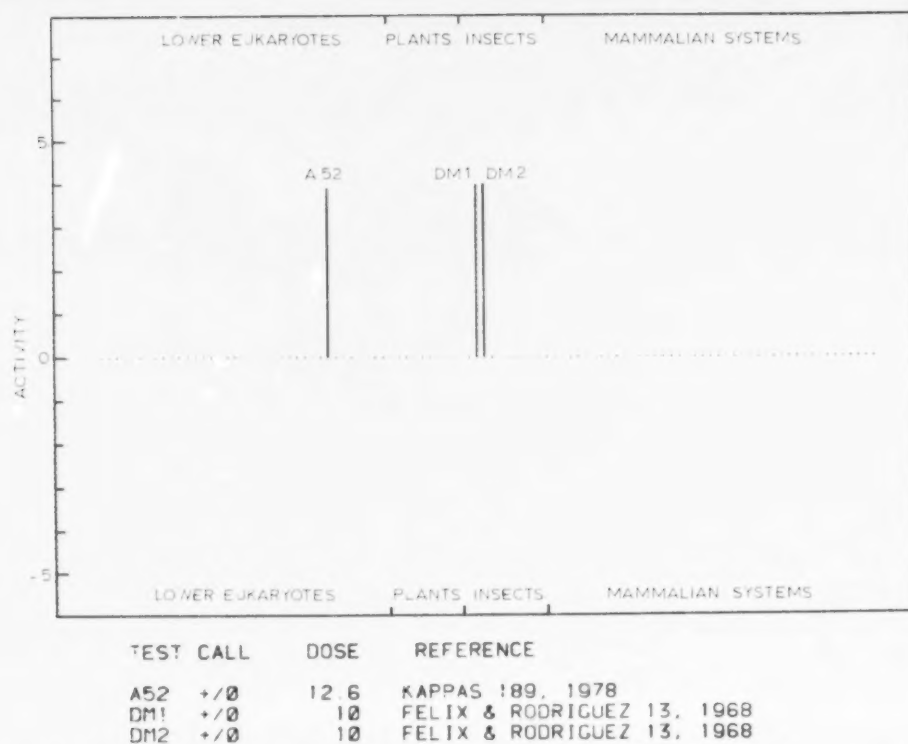


Fig. 8. Activity profile for actinomycin D.

roughly similar dose levels were tested (1000–5000 $\mu\text{g}/\text{ml}$), the reason for such discordant results is not immediately apparent. Benzo[*a*]pyrene gave negative results in *Allium*.

Aspergillus

Aspergillus (Fig. 15) responded positively to relatively low concentrations of benomyl (0.15–0.8 $\mu\text{g}/\text{ml}$) and MBC (0.2 $\mu\text{g}/\text{ml}$). Griseofulvin was found positive in *Aspergillus* at somewhat lower concentrations (4 $\mu\text{g}/\text{ml}$) than in higher plants (150 $\mu\text{g}/\text{ml}$). In general, it appears that alkylating agents such as MMS and cyclophosphamide are active in *Aspergillus* at higher concentrations than are agents such as benomyl, MBC, and griseofulvin. The latter agents may induce aneuploidy in a more direct manner. As mentioned previously, ethanol produces positive results only at very high dose levels, and DMSO gives negative responses.

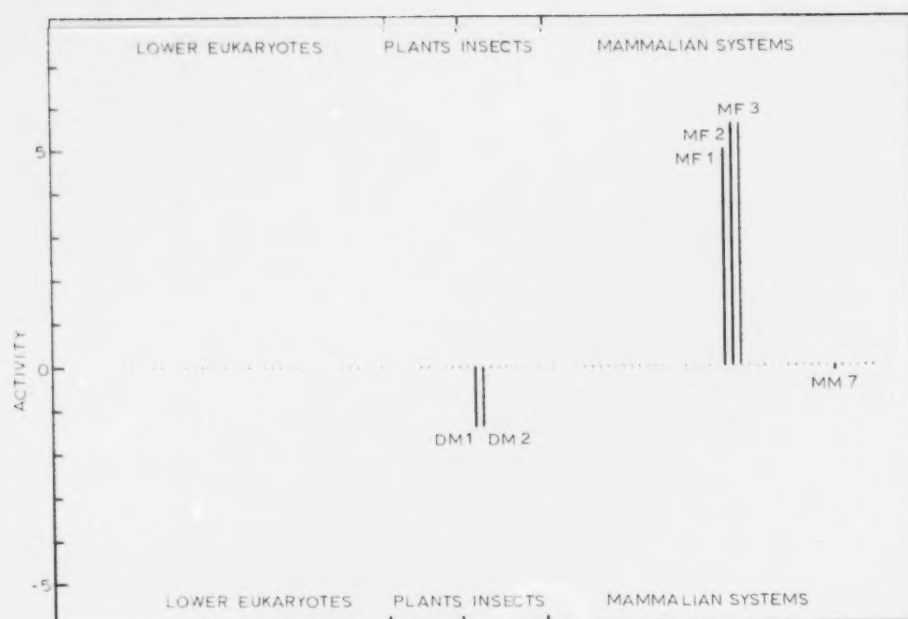
The negative result with bleomycin is somewhat unexpected but may result from the fact that a relatively low dose level (0.5 $\mu\text{g}/\text{ml}$) was tested.

Neurospora

Neurospora (Fig. 16) displays an unusual profile of responses to several chemicals in the meiotic test for disomics. Notable are the negative responses for benomyl, cyclophosphamide, and vinblastine.

Drosophila

In general, meiotic assays for chromosome gain in *Drosophila* (DM2) represent a classic pattern of responses for the agents represented in Fig. 17. The spindle inhibitor colcemid is active in the system at a relatively low concentration (12 $\mu\text{g}/\text{ml}$). Interestingly, the carbamate fungicide benomyl is inactive with moderately high concentra-



TEST CALL	DOSE	REFERENCE
DM1 -/0	23.1	VOGEL 339, 1973
DM2 -/0	23.1	VOGEL 339, 1973
MF1 +/0	1.0	BECKER & SCHONEICH 447, 1982
MF1 -/0	2.0	BASLER 67, 1978
MF2 +/0	0.25	BECKER & SCHONEICH 447, 1982
MF3 +/0	0.25	HANSMANN & ROHRBORN 101, 1973
MM7 -/0	0.25	BINKERT & SCHMID 77, 1977

Fig. 9. Activity profile for triaziquone.

tions (500 $\mu\text{g}/\text{ml}$) having been tested. The triazine herbicide simazine is negative in *Drosophila* when tested at 6000 $\mu\text{g}/\text{ml}$.

Mammalian systems

Mammalian systems are represented collectively in Fig. 18. Mammalian cells in culture responded positively to relatively low doses of the classical spindle poisons colcemid, vinblastine, benomyl (0.02–1 $\mu\text{g}/\text{ml}$), and to higher doses of griseofulvin (40 $\mu\text{g}/\text{ml}$). Benzo[*a*]pyrene also induced aneuploidy at 10 $\mu\text{g}/\text{ml}$ in mammalian cells in the process of in vitro transformation.

Germ cell studies in mammals are very limited.

The positive responses for triaziquone in various female germ cell stages are notable in view of the negative response observed at the same concentration (0.25 mg/kg) in male germ cells. The well-known chemotherapeutic agent cyclophosphamide gives positive results in both female and male germ cells. Also, the anesthetic gas, halothane is negative in female germ cells.

Comparative evaluation of *Neurospora* and *Drosophila*

It was possible to compare results for test systems within different phylogenetic classes (Fig. 19) only in the cases of *Neurospora* (NC1) and

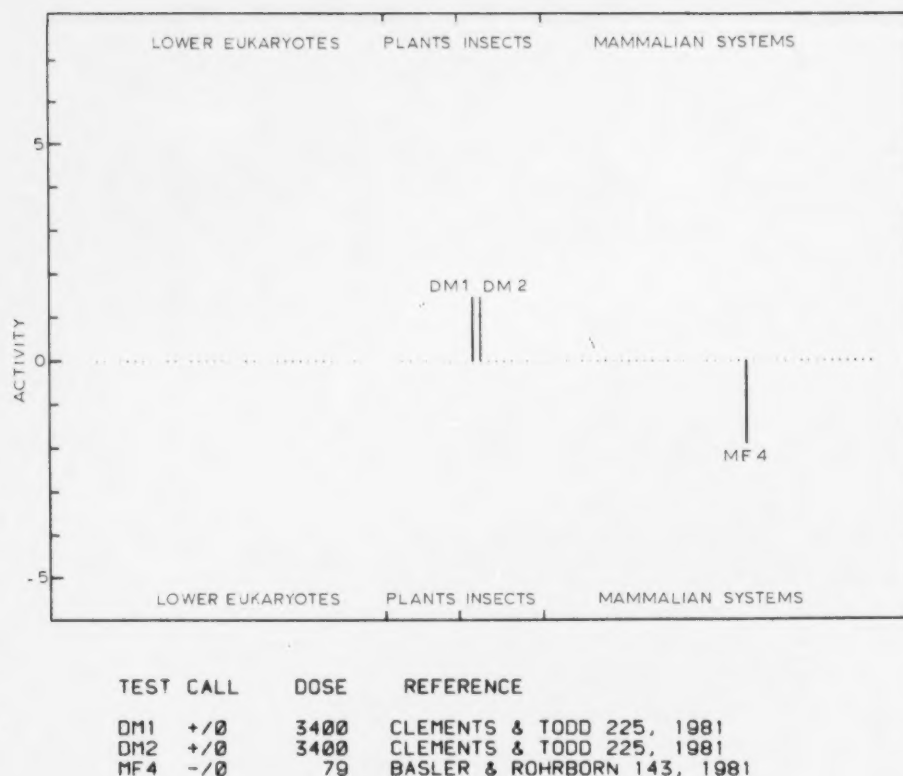


Fig. 10. Activity profile for halothane.

Drosophila (DM2). The assays compared were meiotic ones, and each represented the gain of chromosomes.

8 chemicals were evaluated in both of the assays; results for only 5 of these agents were concordant. The only concordantly positive results were reported with caffeine. 5-Fluorodeoxyuridine and vinblastine were positive in *Drosophila* but negative in *Neurospora*. Atrazine was negative in *Drosophila* but positive in *Neurospora*.

Summary of positive data

Mitotic and meiotic test results may be compared in Fig. 20. Actinomycin D, benomyl, chloral hydrate, colcemid, colchicine, cyclophosphamide, *p*-fluoro-phenylalanine, and vinblastine are reportedly positive in both mitotic and meiotic as-

says. Generally, LEDT values are similar for mitotic and meiotic tests in cases where a chemical has been tested in two or more phylogenetic classes. Of the 20 compounds with three or more test results, DMSO and simazine tested negatively in all systems utilized to measure aneuploidy.

Classification of Compounds Based on Mechanism of Action

In Fig. 21, those compounds displaying results in 3 or more test systems have been placed in rank order by LEDT and have been grouped according to the classification scheme of Bond and Chandley (1983). This scheme subdivides compounds reported to induce aneuploidy into those that: (1) interact with chromosomes and/or DNA; (2) affect tubulin or microtubules or; (3) influence the

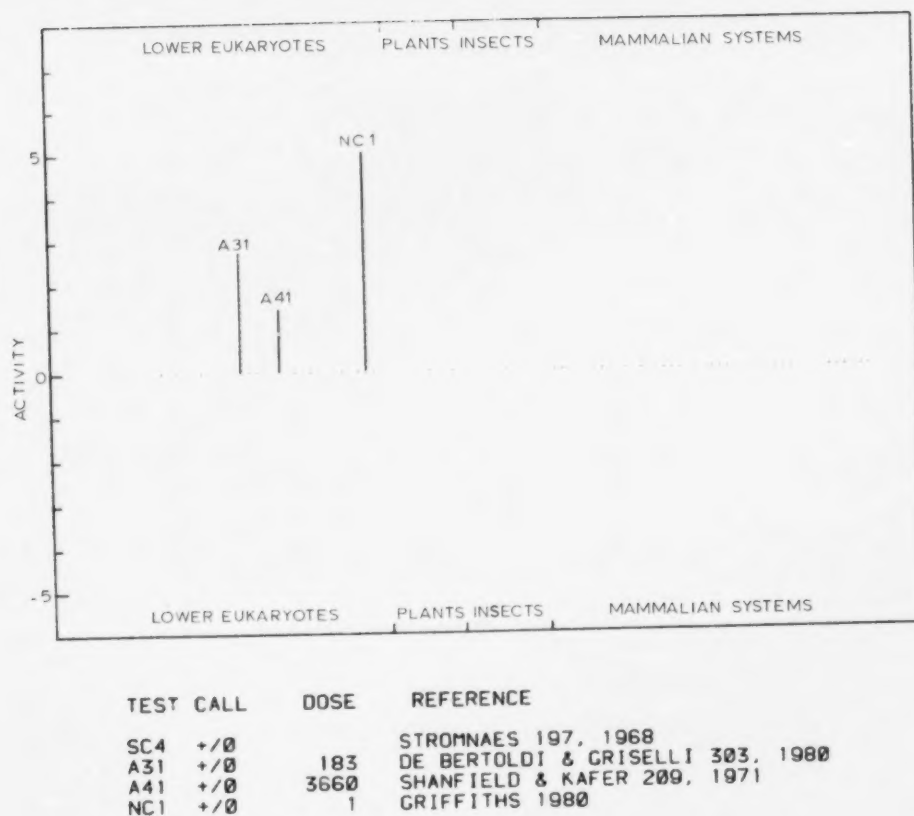


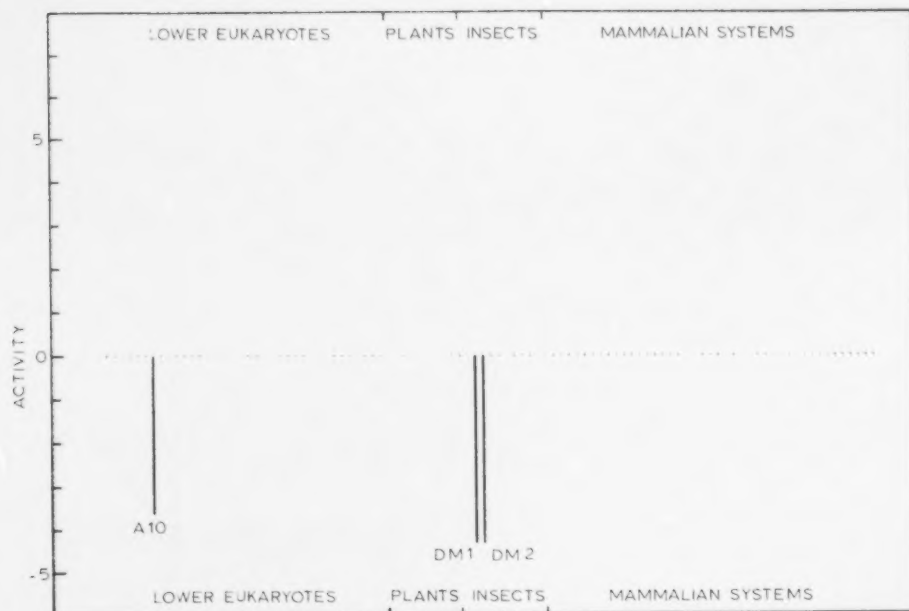
Fig. 11. Activity profile for *p*-fluorophenylalanine.

spindle in other ways. For further discussion on this topic see Bond and Chandley (1983) and the chapter by Liang and Brinkley (this volume).

Conclusion

The foregoing data support the conclusion that there are very few data on the induction of aneup-

loidy in well-defined test systems. To the extent that protocols can be established, further standardization of test systems is required. Clearly, information is required on test system performance against a common set of test agents. Such agents, ideally, would be understood with regard to their mechanism(s) of action.



TEST CALL	DOSE	REFERENCE
A10 -/0	4100	MORPURGO ET AL. 81, 1979
DM1 -/0	20000	TRAUT 273, 1983
DM2 -/0	20000	TRAUT 273, 1983

Fig. 12. Activity profile for dimethyl sulfoxide.

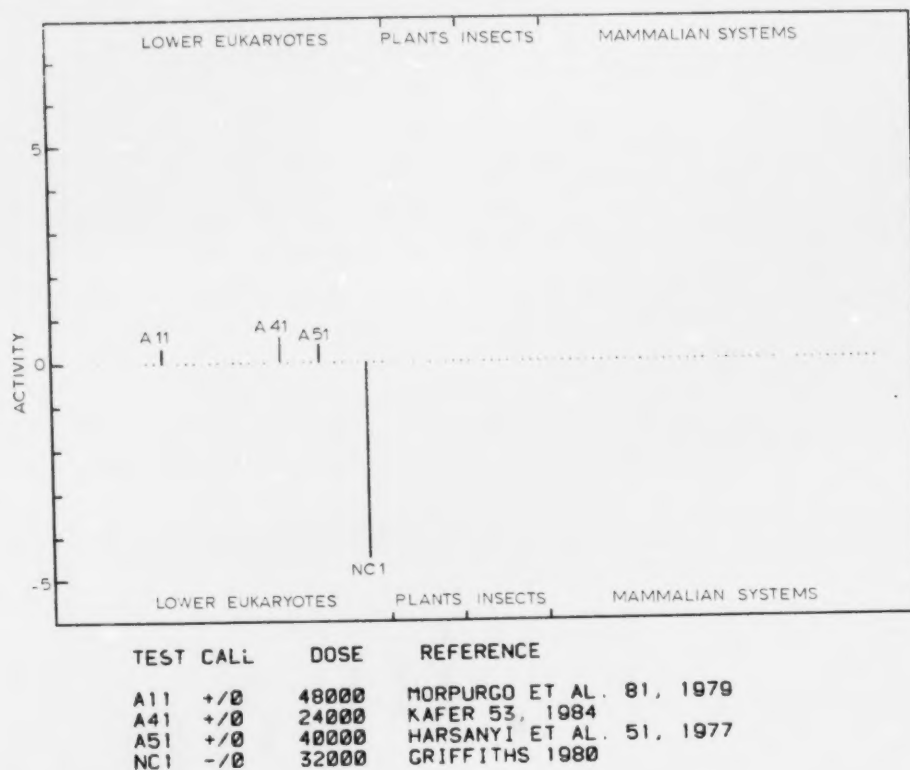


Fig. 13. Activity profile for ethanol.

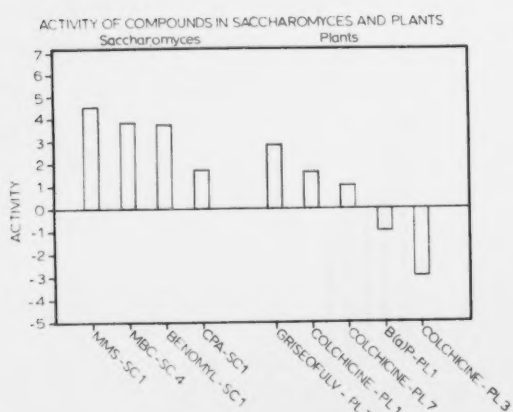


Fig. 14. Activity of compounds in Saccharomyces and plants.

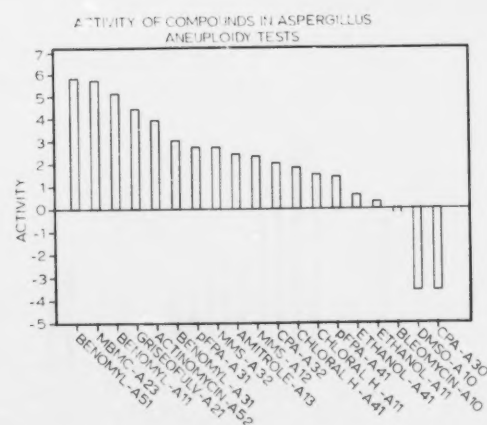


Fig. 15. Activity of compounds in Aspergillus.

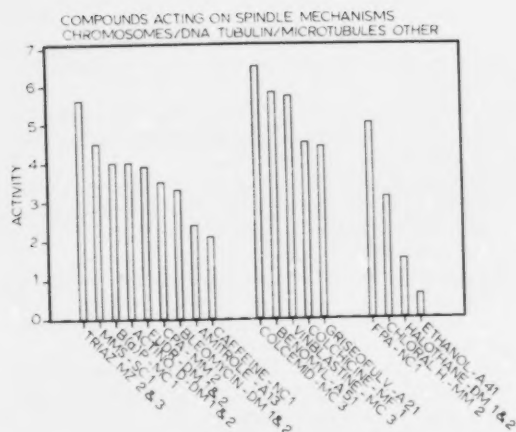


Fig. 21. Classification of compounds based on mechanism of action.

References

- Fig. 21. Classification of compounds based on mechanism of action.

- Lo Schiavo, F.L., V. Nuti Ronchi and M. Terzi (1980) Genetic effects of griseofulvin on plant cell cultures, *Theor. Appl. Genet.*, 58, 43-47.
- Morpurgo, G., D. Bellincampi, G. Gualandi, L. Baldinelli and O.S. Crencenzi (1979) Analysis of mitotic nondisjunction with *Aspergillus nidulans*, *Environ. Health Perspect.*, 31, 81-95.
- Palyi, I. (1976) Mechanisms of spontaneous and induced heteroploidization and polyploidization, *Acta Morphol. Acad. Sci. Hung.*, 24, 307-315.
- Platonova, R.N., and R.A. Svarinskaya (1980) Comparative analysis of long-range mutagenic effect of colchicine in diploid and tetraploid *Crepis capillaris*, *Biol. Bull. Acad. Sci. (U.S.S.R.)*, 7, 179-183.
- Platonova, R.N., V.V. Sakharov, L.I. Katrysh and V.P. Ol'kovenko (1968) Mutagenic aftereffect of colchicine, I. Cytological investigation, *Genetika*, 4(10), 5-13.
- Russo, A., F. Pacchierotti and P. Metalli (1984) Nondisjunction induced in mouse spermatogenesis by chloral hydrate, a metabolite of trichloroethylene, *Environ. Mutagen.*, 6, 695-703.
- Sakharov, V.V., R.N. Platonova, E.F. Melkonova and V.P. Ol'kovenko (1969) Mutagenic aftereffect of colchicine, II, *Genetika*, 5(10), 89-102.
- Schwammenhoferova, K., and M. Ondrej (1978) Induction of chromosomal disturbances other than tetraploidy in barley root tips by colchicine treatment, *Biol. Plant. (Praha)*, 20, 464-467.
- Shanfield, B., and E. Käfer (1971) Chemical induction of mitotic recombination in *Aspergillus nidulans*, *Genetics*, 67, 209-219.
- Singh, M., and U. Sinha (1976) Chloral hydrate induced haploidization in *Aspergillus nidulans*, *Experientia*, 32, 1144-1145.
- Sora, S., G. Lucchini and G.E. Magni (1982) Meiotic diploid progeny and meiotic nondisjunction in *Saccharomyces cerevisiae*, *Genetics*, 101, 17-23.
- Sorsa, M., K. Linnainmaa, M. Penttilä and T. Ilus (1980) Evaluation of the mutagenicity of epoxytrichothecene mycotoxins in *Drosophila melanogaster*, *Hereditas*, 92, 163-165.
- Stromnaes, O. (1968) Genetic changes in *Saccharomyces cerevisiae* grown on media containing DL-para-fluoro-phenyl-alanine, *Hereditas*, 59, 197-220.
- Sugawara, S., and K. Mikamo (1980) An experimental approach to the analysis of mechanisms of meiotic nondisjunction and anaphase lagging in primary oocytes, *Cytogenet. Cell Genet.*, 28, 251-264.
- Tenchini, M.L., A. Mottura, M. Velicogna, M. Pessina, G. Rainaldi and L. de Carli (1983) Double Y as an indicator in a test of mitotic nondisjunction in cultured human lymphocytes, *Mutation Res.*, 121, 139-146.
- Traut, H. (1983) The solvent dimethylsulfoxide (DMSO) does not induce aneuploidy in oocytes of *Drosophila melanogaster*, *Environ. Mutagen.*, 5, 273-277.
- Vogel, E. (1973) Strong antimutagenic effects of fluoride on mutation induction by trenimon and 1-phenyl-3,3-dimethyl-triazine in *Drosophila melanogaster*, *Mutation Res.*, 20, 339-352.
- Waters, M.D., H.F. Stack, K.H. Mavournin and V.L. Dellarco (1985) Special committee report: an evaluation of current testing approaches for the detection of chemically-induced aneuploidy, Part 2. Quantitative evaluation using the genetic activity profile method, in: V. Dellarco, P.E. Voytek and A. Holsaender (Eds.), *Aneuploidy: Etiology and Mechanisms*, Plenum, New York.
- Wood, J.S. (1982) Genetic effects of mythyl benzimidazole-2-yl-carbamate on *Saccharomyces cerevisiae*, *Mol. Cell Biol.*, 2, 1064-1079.
- Zimmermann, F.K., and I. Scheel (1984) Genetic effects of 5-azacytidine in *Saccharomyces cerevisiae*, *Mutation Res.*, 139, 21-24.
- Zimmermann, F.K., V.W. Mayer and I. Scheel (1984) Induction of aneuploidy by oncodazole (nocodazole), an anti-tubulin agent, and acetone, *Mutation Res.*, 141, 15-18.

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